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A method of estimating forage protein degradability is available. In situ neutral detergent fiber nitrogen provides information necessary to calculate metabolizable protein supplied to cattle consuming forage.

Summary

Four experiments including vegetative and dormant forages tested modifications of the in situ neutral detergent fiber nitrogen (NDFN) method of estimating forage undegraded intake protein (UIP). Experiments 1, 2 and 3 tested bag size, closure, rinsing, density, and reflux conditions. None of the modifications affected in situ NDFN content. Experiment 4 compared rates of in situ NDFN digestion calculated with or without correction for undegradability. A close relationship exists between rates calculated by the two methods. Modifications make the improved in situ NDFN method a more desirable means of estimating forage UIP than the standard method.

Introduction

Metabolizable protein, the protein absorbed by the animal, equals the sum of digestible microbial protein and undegraded intake protein (UIP). Information about the ruminal degradability of dietary protein is necessary to describe the contribution it makes to both the microbial protein and UIP. Estimates of DIP and UIP are needed to calculate MP using the 1996 NRC computer software. However, few estimates of forage UIP are available.

Previous research (1997 Nebraska Beef Report, pp. 38-39) indicates neutral detergent fiber nitrogen (NDFN) is an effective method of estimating forage UIP. Our objectives were: 1) to test the effect of modifications of the method on in situ NDFN content; and 2) to examine the relationship between rates of in situ NDFN digestion calculated with or without an undegraded fraction.

Procedure

All three experiments were conducted under similar conditions. Each experiment consisted of one 16- hour incubation in a ruminally fistulated steer fed smooth bromegrass hay (8% CP) at 1.8% of body weight. Smooth bromegrass hay was incubated in every in situ bag. Four bags were incubated for each level of each factor. Estimates of UIP (mg NDFN/g sample incubated) were calculated and each experiment was analyzed separately.

Experiment 1 tested modifications of a standard in situ method. Factors tested were (standard conditions listed first): in situ bag size (10 × 20 cm vs 5 × 10 cm), degree of post-in situ hand rinsing (45 min vs 15 min), bag closure

method (rubber band around a #8 rubber stopper vs heat-sealing) and NDF method (individual refluxing of subsampled residue versus bulk refluxing of the bag containing its residue). The amount of sample incubated in the small bags was reduced (1.25 g) in order to maintain the same sample/bag surface area ratio as the large bags. The same number of rubber stoppers was placed in each mesh bag in order to ensure similar weights for each. A bulk refluxing apparatus was used for neutral detergent extraction (Ankom, Inc., Fairport, NY).

Experiment 2 was conducted using modifications tested in Experiment 1. All in situ bags were 5 × 10 cm and contained 1.25 g of sample. Bags were hand-rinsed for 15 min immediately after the incubation. The bags were heat-sealed and refluxed in neutral detergent solution in the bulk apparatus. The first factor tested a modification of the standard incubation conditions. In situ bags were incubated in the steer at two densities (20 bags/mesh bag versus 50 bags/mesh bag). Two mesh bags were incubated for each density. The second factor concerned the refluxing conditions. Use of the bulk reflux apparatus required placement of the in situ bags in a cylindrical rack. The rack consists of eight removable dishes. Each dish is capable of holding three in situ bags, for a total of 24 bags possible in each reflux. Dishes were stacked vertically and held by a metal rod. We hypothesized dish position would not affect NDFN content. The top and bottom two racks were used as the two levels of the second experimental factor. Twelve bags from each mesh bag density (three per replication) were chosen randomly and allotted randomly to either the top or bottom level of the two conducted refluxes.

Experiment 3 was conducted using modifications tested in Experiment 2. Small, heat-sealed in situ bags (5 ×10 cm) were incubated at a density of 50 bags/mesh bag. Bags contained 1.25 g of sample and were rinsed after incubation for 15 min. Bags were assigned randomly to dishes in the bulk reflux rack. Factors tested were time of NDF reflux (40 min vs 70 min) and extent of post-NDF bag rinsing (.5 L water/bag vs 1 L water/bag).

Experiment 4 was conducted to describe the effect of correcting the rate of in situ NDFN digestion (k_d) for an undegradable fraction. Three sets of forage samples were incubated together in the rumen and were collected using either esophageally or ruminally fistulated animals. Samples were collected from animals grazing the following forages (number of samples taken in parentheses): cornstalks (n=24), growing cool-season grasses (n=36) and winter native range (n=24).

All of Experiment 3's incubation modifications were used. Small bags (5 x 10 cm) were heat-sealed and incubated at a density of 50 bags/mesh bag. Bags were refluxed in neutral detergent solution in groups of 24. Each sample was incubated for 2, 12 or 96 hours. Incubations were replicated three times. Two different regression equations were calculated using the natural logarithm of mg NDFN/g of sample incubated. The slope of the regression equation equals k_d. The first k_d was calculated using bags incubated for 2 and 12 hours. This method assumes that NDFN is 100% degradable in the rumen. The second k_d was calculated by subtracting the 96 hour (96NDFN) value from both the 2 and 12 hour values. These new values were used to calculate a separate k_d. The second method assumes that the NDFN pool has reached its extent of ruminal degradation by 96 hours. Regression analysis was used to describe the relationship between the two calculations.

Results

No differences (P>.05) in 16-hour in situ NDFN content were observed between bag sizes. These results agree with previous research, which indicated forage DM digestibility is unaffected by bag size as long as the sample size/bag surface area ratio remains constant.

Rinsing in situ bags after incubation is necessary for the removal of rumen microbes from the bag and its residue. It was hypothesized that less rinsing would be necessary if NDFN was the UIP pool. Previous results indicate neutral detergent solution reflux removes attached microbes *(*1997 Nebraska Beef Report, pp. 38-39*)*. No differences (P>.05) were observed in 16-hour in situ NDFN content between bags rinsed for 45 versus 15 min. Reduction in the time spent washing makes the method more efficient and might reduce washout of small particles.

The final two factors in Experiment 1 (method of bag closure and NDF method) were included to test the efficacy of bulk refluxing of bags. It is necessary to heat-seal the bags when reflux is conducted directly on the bag and its residue. No effect (P>.05) of either factor was found. The results of Experiment 1 indicate the in situ NDFN procedure can be conducted using smaller, heat-sealed bags rinsed for 15 min after incubation and refluxed in bulk. These modifications will decrease the amount of labor needed to conduct the procedure.

The standard in situ method allows no more than 20 in situ bags in one mesh bag and up to 6 mesh bags in one ruminal incubation. However, the use of smaller bags may allow a greater mesh bag density to be used. No effect (P>.05) of mesh bag density was observed. Therefore, up to 50 in situ bags can be placed in one mesh bag and up to 300 in situ bags (5×10 cm) can be incubated in a large, fistulated bovine. Similarly, no effect (P>.05) was found for position of bags within the bulk refluxing apparatus. Bags may be allotted randomly to any dish in the bulk reflux rack without affecting NDFN content. No differences (P>.05) between reflux times or extent of post-neutral detergent extraction rinsing were observed. These results imply reflux time and extent of rinsing are not critical to the method.

Previous estimates of in situ NDFN UIP assume NDFN is 100% digestible in the rumen. However, this assumption is inconsistent with cell wall digestion models, which assume ruminally undegradable fiber exists. It is important to have an accurate estimate of k_d for a UIP fraction. The purpose of Experiment 4 was to describe the effect

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Table 1. Regression equations for correcting the rate of in situ neutral detergent fiber nitrogen (NDFN) digestion for an undegradable fraction.

Sample Set	n	Equation	r^2
Native Winter Range	24	$.468 + 1.174X + .023X^2$	.952
Cornstalks	24	$.584 + .956X + .035X^2$	.946
Vegetative Cool-Season Grasses	36	$.176 + 1.221X + .051X^2$	.804
Combination of all sets	84	$.400 + 1.227X + .028X^2$	.854

X = uncorrected rate of digestion calculated from 2 and 12-hour in situ NDFN content

of correcting the NDFN k_d for an undegraded nitrogen fraction. The amount remaining after 96 hours was assumed to be undegradable in the rumen.

Regression equations describing the effect of correcting for an undegradable UIP fraction are shown in Table 1. The equations explain a high proportion of the variation in NDFN k_d (i.e. $r^2 \geq .80$). Equations for cornstalks and native winter range were not statistically different.These results imply a close relationship exists between the two methods of calculating k_d. When equations are developed for a particular forage type at a location, corrected NDFN UIP values can be estimated from uncorrected values using the prediction equation.

In summary, the results of Experiments 1, 2, 3 and 4 imply all tested modifications can be implemented into an improved method. Such a method will save time and money relative to the standard in situ procedure and will provide more accurate estimates of forage protein degradability. Information obtained by this method will contribute to more accurate use of the 1996 NRC Beef Cattle software.

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